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New Family of Neutralizing Antibodies in HIV Asymptomatic Long-Term Non-Progressors

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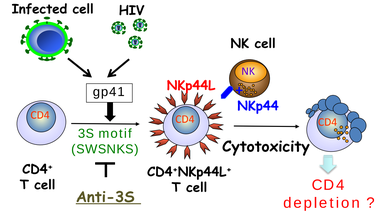
Abstract

Background: Through a large serie of studies, we previously characterized the pathogenic effect of the highly conserved 3S epitope of gp41 during HIV infection. By analyzing the humoral immune response induced in asymptomatic long-term non-progressor (ALT) patients, we recently observed that up to 25% patients elicited efficient neutralizing responses directed against a point-mutated form of 3S (W614A-3S). Here we extensively characterized the neutralizing profile of this new family of neutralizing Abs.
Methods: Abs binding to 3S-WT (wild-type) or W614A-3S mutants were isolated from the sera of ALT patients. Abs were purified from heat-inactivated plasma of ALT patients by immunoprecipitation with synthetic W614A-3S peptides immobilized onto an amine-reactive agarose support, concentrated with Centicon filter, and dialyzed against PBS. The functional inhibitory profile of these Abs was defined using the well-standardized TZM-bl neutralization assay, the conventional neutralization assay on PBMCs, or the Fc-mediated inhibitory assay on macrophages.
Results: We found that the anti-W614A-3S purified Abs display efficient and broad neutralizing activity. They inhibit transmitted founder Tier 2 viruses, neutralize primary isolates on primary cells and display Fc-mediated inhibitory functions at ng to µg/ml concentrations. The detection of anti-W614A-3S Abs was specifically correlated both with lower viral DNA (p<0.0001), viral load (p<0.0001), and other clinical parameters (CD4⁺ T cells, HLA protective alleles, …) suggesting that anti-W614A-3S neutralizing Abs participate in the control of HIV replication in ALT patients.
Conclusion: These results demonstrate that ALT patients develop efficient neutralizing Abs that can be purified using W614A-3S mutant protein capture assays. These Abs are distinct to that recently isolated from ELITE neutralizer patients. Abs directed against W614A-3S may therefore be considered as a new family of broadly neutralizing Abs, which need to be further characterized, considering their potential role on viral load and viral DNA.

Introduction

Over the ensuing years, and through a series of published data, we have developed a new vaccine strategy based on a highly specific and conserved motif, called 3S, located in the gp41 HIV-1 protein. This highly pathogenic motif induces expression of NKp44L, the cellular ligand of an activating NK receptor (NKp44) (Baychelier et al *Blood* 2013), rendering CD4⁺ T cells more sensitive to NK lysis (**Figure 1**).



An alanine-scanning allowed us to identify specific positions in the 3S motif that inhibit HIV entry and expose the highly conserved motif to a broad spectrum of NAbs. A Specific substitution in the 3S, called W614A-3S, was clearly associated with both impaired infectivity and virus entry. In addition, we showed for the first time, that specific anti-W614A-3S Ab elicit broadly neutralizing activity against HIV-1 (Petitdemange *et al Clin Infect Dis* 2013). Anti-W614A-3S Nabs have been generated in mouse and rabbit, but also naturally detected in less than 5% HIV-1 progressor patients. In the present study, we investigated the presence of W614A-3S neutralizing Abs in untreated long-term non progressor (LTNP) where HIV infected people maintain high CD4⁺ T-cell counts and remain therapy naïve.

Methods

ALT patients : 71 patients have been recruited for the French National Cohort of Long Term Non Progressors (LTNPs), designated as ALT (for Asymptomatiques à Long-Terme) ANRS CO15 cohort, on the basis of a known history of HIV-1 infection for at least 8 years, the absence of antiretroviral therapy and a CD4⁺ T cell count above 600 cells/ml, whatever their levels of viral load and viral DNA.

Elisa : The anti-3S/WT and anti-W614A-3S Abs from sera of ALT patients were titrated by enzyme-linked immunosorbent assay (ELISA). A pool of human AB serum from healthy donors was used as negative control. Quantification of anti-gp41 Abs used calibration standards including dilution of exact concentrations of purified mouse anti-3S mAb. Antibody amounts were expressed in arbitrary units (AU). This test has a detection limit of 10 AU/mL.

Neutralization assay : Specific Abs were purified from heat-inactivated plasma of ALT patients by immunoprecipitation. The Pierce direct IP kit was used , and synthetic 15-mer 3S (WT and W614A) peptides were directly immobilized onto an amine-reactive agarose support. Purified Abs were dialyzed against PBS, quantified, and then tested at starting concentrations of 2 µg/ml, followed by 2-fold dilutions.

Neutralization of purified Abs was tested using the standard Tzm-Bl assay, or with TZMbl cells expressing FcgRI receptor, at 48 hr post-infection. A cutoff value of 2.5 times background was applied to determine positive values. Neutralization assays were performed against tiers 1 and tiers 2 clade B HIV-1 strains (ie NL4.1, NDK, SF162, JR-CSF, YU-2, QHO).

Concomitantly to the classical TZM-bl standard assay, the capacity of W614A-3S Ab to inhibit HIV-BaL infection was assessed in monocyte-derived macrophages (MDM)

High frequency and absence of clinical evolution of ALT patients producing anti-W614A-3S Abs

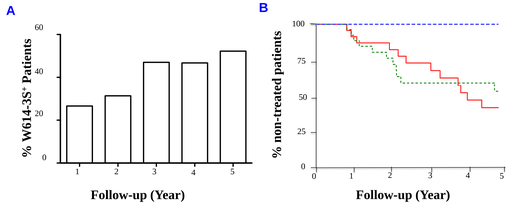


Figure 2 : (**A**) Increased proportion of patients producing W614A-3S Ab during the follow-up. (**B**) Kaplan-Meier curves on the frequency of non-treated patients in ALT patients, which do not produce anti-3S Abs (Neg; (n=24, yr1), only produce anti-WT-3S Abs (WT-3S⁺; n=28, yr1), or produce anti-W614A-3S Abs (W614A-3S⁺; n=16, yr 1).

Anti-W614A-3S Abs produced in ALT patients elicit viral neutralization against tier 1 and tier 2 HIV-1 strains

Samples	Year	IC_{80} (µg/mL)								
		TZM-bl assay						TZM-bl/FcγRI assay		MDM assay
		NL4.3	NDK	SF162	JR-CSF	YU-2	QHO92	SF162	QHO92	BaL
02.002	1	0.7	2	>5	3	>5	>5	1.2	>5	0.1
	2	0.5	1	0.3	1	5	3	<0.3	1	0.001
	5	0.1	0.5	0.3	0.3	1	3	<0.3	1.2	0.005
	8	0.5	1	>5	>5	>5	>5	5	>5	0.1
	10	2	5	>5	>5	>5	>5	5	>5	0.2
04.034	1	0.7	1	5	3	>5	>5	0.3	5	0.05
	2	<0.1	0.1	0.5	1	5	4	<0.3	<0.3	0.005
	5	<0.1	<0.1	<0.3	<0.1	0.3	0.7	<0.3	0.3	0.008
	10	<0.1	<0.1	0.3	<0.1	0.1	1	0.3	0.4	0.05
09.015	1	1	3	>5	5	>5	>5	<0.3	0.3	0.1
	2	0.1	1	0.3	1	3	2	1	>5	0.08
	5	<0.1	<0.1	<0.3	<0.1	0.2	0.4	<0.3	0.7	0.05
	10	0.5	1	0.3	1	5	3	<0.3	0.3	0.05
	12	2	3	>5	>5	>5	>5	<0.3	0.6	0.03
3S-WT		>10	>10	>10	>10	>10	>10	>10	>10	>10

Table 1 : Neutralization profiles of purified W614A-3S Ab from serum of three patients (02.002, 04.034 and 09.015) at different time-points of the follow-up (ranged between 1 to 12 years) in assays with TZM-bl, TZM-bl/FcγRI, or monocyte-derived macrophages (MDM). A orange-color scale indicates ranges of values for maximal concentration needed to 80% of virus inhibition (IC_{80}).

Lower viral load and viral DNA in ALT patients with anti-W614A-3S neutralizing Abs

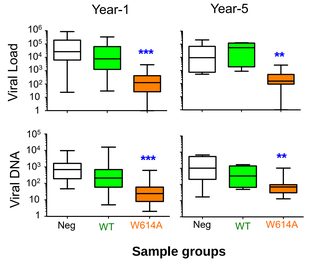


Figure 3 : Viral load and viral DNA levels in ALT patients, which do not produce anti-3S Abs (Neg), only produce anti-WT-3S Abs (WT), or produce anti-W614A-3S Abs (W614A).

Association between viral load, viral DNA and neutralizing activity in ALT patients with anti-W614A-3S Ab

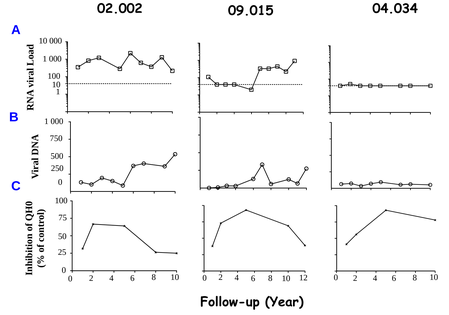


Figure 4: RNA viral load (**A**), Viral DNA (**B**) and neutralizing activity (**C**) in 3 different ALT patients producing W614A-3S Ab (02.002, 099.015 and 04.034). Neutralizing activity was obtained in the TZM-bl assay with HIV-1 QH0 virus. Similar data were obtained with SF162, YU-2, and JR-CSF viruses (data not shown).

High CD4⁺ T cell count and CD4 responses in ALT patients with anti-W614A-3S Abs

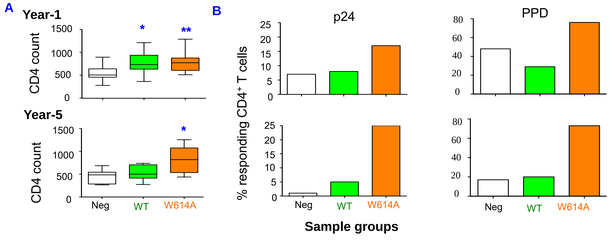


Figure 5 : (**A**) CD4⁺ T-cell count and (**B**) frequency of CD4⁺ T-cell responses against HIV-p24 and PPD in proliferation assay after ^{3}H-thymidin incorporation. Experiments were performed, at year 1 and 5 of the time-course, in ALT patients in ALT patients, which do not produce anti-3S Abs (Neg), only produce anti-WT-3S Abs (WT), or produce anti-W614A-3S Abs (W614A).

Conclusion

We found that polyfunctional W614A-3S Abs strongly correlated with low viral load, low viral DNA, and high CD4⁺ T cell responses. This suggests that W614A-3S Ab could be associated with the LTNP status.